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3. "cis-urocanic acid is not useful as an immunosuppressive agent in the treatment of human allergic contact dermatitis", Kammeyer et all, Archives of dermatological research, 1996, 288 (11), 725-7.

Brian Kwon
REM 4B81
Patent Examiner, Pharm-D.
(571)272-0581

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Rapid Report

Urocanic acid isomers are good hydroxyl radical scavengers: a comparative study with structural analogues and with uric acid

Arthur Kammeyer [a,*], Teunis A. Eggelte [b], Jan D. Bos [a], Marcel B.M. Teunissen [a]

[a] *Department of Dermatology, Academic Medical Centre, P.O. Box 22660, 1100 DD Amsterdam, Netherlands*
[b] *Department of Clinical Pharmacology, Academic Medical Centre, P.O. Box 22660, 1100 DD Amsterdam, Netherlands*

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Abstract

UV-exposure of the epidermis leads to the isomerisation of *trans*-UCA into *cis*-UCA as well as to the generation of hydroxyl radicals. This study shows by means of the deoxyribose degradation test that UCA isomers are more powerful hydroxyl radical scavengers than the other 4-(5-)substituted imidazole derivatives, such as histidine, though less powerful than uric acid. UCA, present in relatively high concentrations in the epidermis, may well be a major natural hydroxyl radical scavenger.

Keywords: Deoxyribose degradation; Histidine; Hydroxyl radical; Imidazole; Uric acid; Urocanic acid

Trans-urocanic acid (*trans*-UCA) is a major ultraviolet (UV) absorbing component of the human epidermis. Absorption of UV radiation from the UV-C region (200–290 nm) into the UV-A-I region (340–400 nm) causes photoisomerisation of *trans*-UCA into *cis*-UCA in vivo as well as in vitro [1–3]. Because of this property, *trans*-UCA had been used as a natural sunscreen agent [4]. This use had later been minimised since it became clear that photoproduct *cis*-UCA can mimic some of the effects of UV on immunity, suggesting that this compound is an important mediator of UV-induced immunosuppression [5]. UV exposure of the skin causes an increased level of oxidative stress with the inherent formation of reactive hydroxyl radicals [6]. These species can be generated from hydrogen peroxide upon UV irradiation and upon contact with metal ions (e.g. ferrous ions, Fe^{2+}), the Fenton reaction. Both types of reaction can occur in the epidermis [7]. Under these conditions, UCA isomers may interact with the randomly produced hydroxyl radicals in situ. In this study, we tested in vitro the hydroxyl radical scavenging ability of both UCA isomers, of chemically related compounds, and of the known scavenger uric acid. The results of this comparative study point to certain molecular structures required for good hydroxyl radical scavenging ability and provide a ranking of *trans*-UCA and *cis*-UCA among other (known) scavengers.

Trans-UCA, *cis*-UCA, L-histidine, dihydrourocanic acid [3-(imidazol-4-yl)propionic acid], imidazole-4-acetic acid (sodium salt), imidazole, 2-methylimidazole, L-alanine, *trans*-2-furylacrylic acid (*trans*-FAA) and uric acid (Fig. 1) were tested on their ability to scavenge hydroxyl radicals by means of the deoxyribose (dR) degradation test. Upon expo-

* Corresponding author. Fax: +31-20-566-4440; E-mail: a.kammeyer@amc.uva.nl

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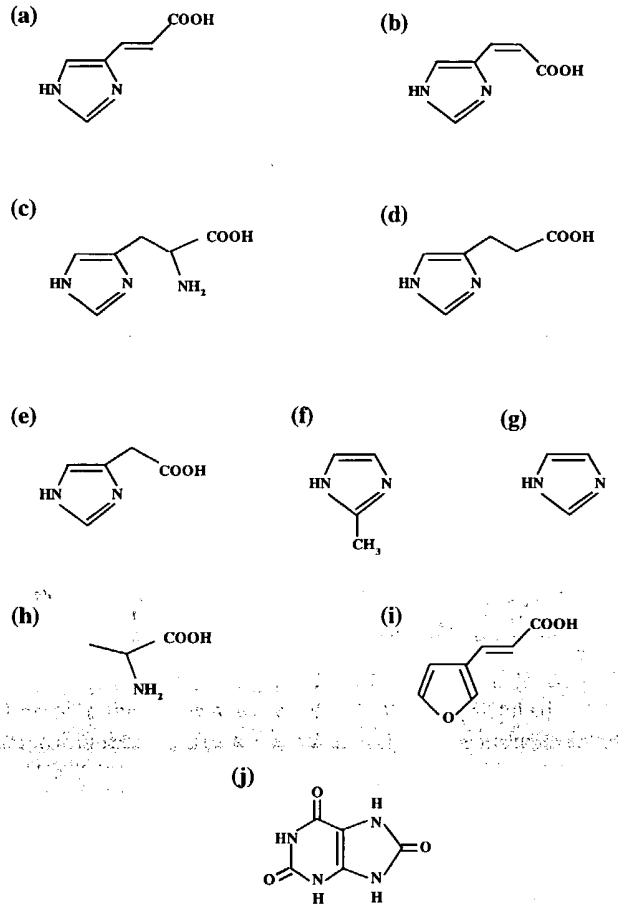

Fig. 1. Compounds tested in this study for hydroxyl radical scavenging ability. (a) *trans*-UCA, (b) *cis*-UCA, (c) L-histidine, (d) dihydro-UCA or 3-(imidazol-4-yl)propionic acid, (e) imidazole acetic acid, (f) 2-methylimidazole, (g) imidazole, (h) L-alanine, (i) *trans*-2-furylacrylic acid and (j) uric acid.
←

sure to hydroxyl radicals dR is degraded into malondialdehyde, which reacts with thiobarbituric acid to form a pink chromogen. Hydroxyl-radical scavengers will compete with dR, resulting in a reduced amount of malondialdehyde. This dR degradation test was analogous to an earlier described method [8]. Briefly, the reactions were performed in 5-ml screw-cap glass tubes in a final volume of 1.0 ml sodium phosphate buffer (50 mM; pH 7.2), containing 3.0 mM 2-deoxy-D-ribose, 0.5 mM hydrogen peroxide and one of the test compounds at graded concentrations. The reaction was started by the addition of premixed disodium EDTA and ferrous iron solutions (final concentrations 0.5 and 0.2 mM, respectively). Ferrous ammonium sulphate served as source for ferrous ions (Fe^{2+}). Fe^{2+} solutions were freshly prepared each time and were purged with nitrogen. The mixture was left for 15 min at room temperature. After addition of 1.0 ml 1% thiobarbituric acid in 50 mM sodium hydroxide and 0.75 ml 2.8% trichloroacetic acid, the tubes were heated for 20 min in a boiling water bath. The pink colour was read at 532 nm and

Table 1
The hydroxyl radical scavenging ability of urocanic acid isomers and related compounds

Hydroxyl radical scavenger	Second-order rate constant $\times 10^9$			Inhibition of deoxyribose degradation [scavenger] = [deoxyribose] = 3 mM
	M^{-1} s^{-1}	S.D.	n[b]	(%)
Imidazoles				
***Trans*-urocanic acid**	**8.0**	0.9	8	**67**
***Cis*-urocanic acid**	**7.1**	0.6	6	**64**
L-Histidine	2.6[c]	0.9	4	34
Dihydrourocanic acid	2.7	0.9	3	34
Imidazole-4-acetic acid	2.2	0.1	3	30
Imidazole	13.0	0.9	5	78
2-Methylimidazole	11.7	2.6	5	76
Other compounds				
L-Alanine	0.1	0.0	3	2
Trans-2-furylacrylic acid[a]	< 0.1	–	3	< 2
Uric acid	27.8	3.0	4	91

[a] *Trans*-2-furylacrylic acid was not tested in concentrations > 8 mM because of poor solubility.
[b] n represents the number of slopes from which the rate constant was calculated.
[c] $2.3–3.0 \times 10^9$ M^{-1} s^{-1} in literature [8].

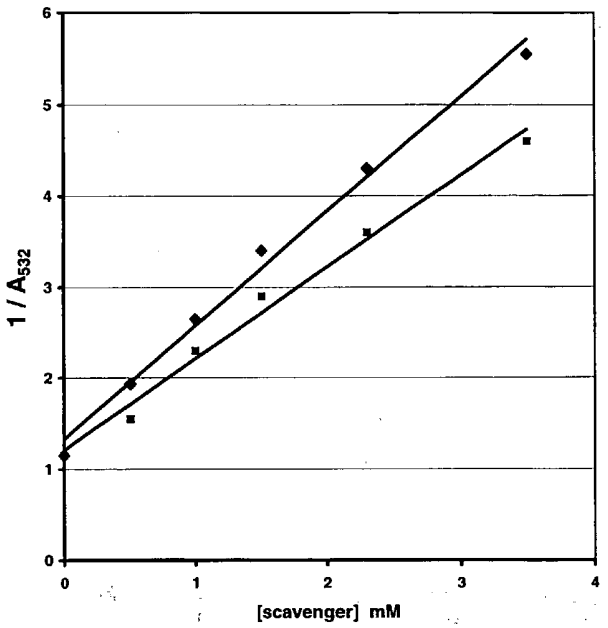

Fig. 2. A determination of the second order rate constants of *trans*-UCA (◆) and of *cis*-UCA (■) with hydroxyl radicals. The rate constant was derived from the slope of the line (k = slope × k_{dR} × [dR] × A_0), where A_0 is the absorbance, measured in the absence of hydroxyl radical scavenger. k_{dR} was taken as 3.1×10^9 M^{-1} s^{-1}, derived from pulse radiolysis studies [8], and [dR] = 3 mM. The rate constants in this particular set were 8.49 and 7.33×10^9 M^{-1} s^{-1} for *trans*-UCA and *cis*-UCA, respectively. The other scavengers were studied similarly.

reciprocal absorption values were plotted against the concentration of the test compound after subtraction of appropriate blanks. A series of six duplicate determinations from test compound dilutions was employed to construct a graph slope for the calculation of a rate constant value. A typical graph with slopes to derive rate constants from is shown in Fig. 2 for both UCA isomers. The mean, S.D., number of rate constants and the percentage of inhibition of deoxyribose degradation at equimolar concentrations of scavenger (3 mM) is calculated for each test compound and summarised in Table 1.

Trans-UCA and *cis*-UCA are substantially stronger in scavenging hydroxyl radicals (8.0 and 7.1×10^9 M^{-1} s^{-1}, respectively), than the other 4-(5-)substituted imidazoles, including L-histidine (2.6×10^9 M^{-1} s^{-1}). Histidine, the precursor of UCA, was included as a known scavenger [8–10] with structural similarities to UCA. Alanine was used as a known poor scavenger [10]. *Trans*-FAA was tested as a non-imidazole acrylic acid derivative, having a furan ring instead. This substitution yielded a very poor scavenging ability. Other 4-(5-)substituted imidazole analogues, dihydrourocanic acid or 3-(imidazol-4-yl)-propionic acid and imidazole-4-acetic acid, showed moderate scavenging ability, comparable to histidine. However, unsubstituted imidazole and its 2-methyl derivative appeared to be stronger scavengers than the UCA isomers.

The known strong hydroxyl radical scavenger uric acid [11] showed an excellent scavenging ability (27.8×10^9 M^{-1} s^{-1}). To summarise, *trans*-UCA and *cis*-UCA, two epidermal compounds, are good hydroxyl radical scavengers. Their scavenging ability is weaker than that of uric acid, but larger than the other 4-(5-)substituted imidazoles, e.g. histidine.

Normal human skin contains approximately 200 μM iron [12,13], predominantly complexed to ferritin. The release of free ferrous ions by UV irradiation [14] and the presence of hydrogen peroxide [15,16] are prerequisites for the generation of hydroxyl radicals. Other reports indicate the UV-induced presence of hydroxyl radicals indirectly since their effects on epidermal constituents could be neutralised with antioxidants [17,18].

UCA is an imidazole compound and several other imidazole derivatives have already been shown to be good hydroxyl radical scavengers, e.g. histidine [8–10], histamine [19], histidine containing dipeptides [10,20], cimetidine and other histamine (H_2) receptor antagonists [21]. This study reveals that several other imidazoles show similar properties (Table 1). Hydroxyl radicals can react with the imidazole ring to form imidazolone derivatives. Their formation has led to the proposal to use the imidazolones of histidine and histamine as markers for oxidative stress [9,19]. The importance of the imidazole ring in UCA molecules was also demonstrated in our experiments. The poor scavenging ability of *trans*-FAA, having a furan ring instead, was a remarkable contrast. Furthermore, the presence of the acrylic acid moiety in UCA molecules conjugated with the imidazole ring may account for its increased scavenging ability towards hydroxyl radicals as compared to the other 4-(5-)substituted imidazoles. Unsubstituted imidazole and its 2-methyl derivative are stronger hydroxyl radical scavengers, accentuating that the presence of an imidazole ring is a prerequisite for

sufficient hydroxyl radical scavenging ability. However, these compounds do not occur physiologically and are harmful (LD_{50} oral rat 220 mg kg^{-1} for imidazole and 1500 mg kg^{-1} for 2-methylimidazole).

Two explanations for the relatively high concentration of UCA in the epidermis have already been put forward: (1) for *trans*-UCA as natural sunscreen agent; and (2) for *cis*-UCA as immunosuppressant. Our findings point to another physiological role for the UCA isomers. *Trans*-UCA and *cis*-UCA may be major natural hydroxyl radical scavengers, providing a new view on the antioxidant status of the skin.

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